FINAL REPORT FOR COSMOS 2044

K-7-03: GRAVITY AND SKELETAL GROWTH

Emily R. Morey-Holton NASA-Ames Research Center Moffett Field, CA

Stephen B. Doty
Hospital for Special Surgery
New York City, NY

W. Eugene Roberts, Larry P. Garetto Indiana University School of Dentistry Indianapolis, IN 46202

Nicola C. Partridge
St. Louis University School of Medicine
St. Louis, MO

Robert T. Whalen
NASA-Ames Research Center
Moffett Field, CA

Alan Hargens
NASA-Ames Research Center
Moffett Field, CA

Soviet Collaborators: **G. Durnova, A. Kaplansky**Institute of Biomedical Problems
Moscow, USSR

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## SUMMARY

Bone area and perimeter were measured at the tibiofibular junction of rats associated with Cosmos 2044. Bone vascularity and bone cells within the tibial diaphysis, and collagen fibrils in the tendons of the foot were studied at the light and electron microscope level. Investigations on progenitor populations, collagenase activity in the calvaria, biomechanical properties of the tarsals, are not yet complete. Thoracic, rather than lumbar, vertebra were received but the disks were not large enough for measurement of disk swelling properties.

No major differences were noted between any of the groups. The only significant difference was the larger marrow parimeter in the basal rats as compared to all other groups. The rats were about 25d older than the Cosmos 1887 animals. The data suggest that few bone changes occur in rats launched into space at 107d of age for 14 days.

# INTRODUCTION

Experiments flown on Soviet Cosmos biosatellite 1887 were complicated by the unexpected postflight processing time which was in excess of two days after landing of the satellite. To differentiate between flight response with minimal recovery time and flight response with extended recovery superimposed, Cosmos 2044 was launched and the experiments previously flown on Cosmos 1887 were repeated. However, due to unanticipated flight delays, the rats on Cosmos 2044 were almost one month older than the animals on Cosmos 1887 at launch. Cosmos 2044 was also the first flight experiment to include the ground-based flight-simulation rat model (tail-suspended animals) as a control group.

## MATERIALS AND METHODS

Specific pathogen-free, male, Wistar rats from the Institute for Experimental Endocrinology of the Slovakian Academy of Sciences were divided into 4 groups of 10 animals/group and a baseline control group

of 5 rats. The flight and synchronous groups were each housed in a single cage which had 10 nozzles for paste diet and 10 lixits for water. Fourteen gram boluses of food (55g/day/nozzle) were delivered every 6 hours beginning at 0200 each day. Water was provided ad libitum. The vivarium animals were housed similarly but were fed in a single bolus each day. The flight rats were launched on September 15, 1989, at 1030 hours and landed at 0600 hours on September 29, 1989. Rats #6-10 were used in this experiment. The flight rats were 14 days older than the basals while the vivarium rats were 6 days older than the flight and the synchronous group was 2 days younger than the vivarium group at the end of the experiment. At the end of each test period, rats were guillotined. One-half of the proximal tibial metaphysis, the tibial shaft, and one skinned foot were place in vials of 2% parformaldehyde in 0.1M cacodylate buffer, plus 0.5% glutaraldehyde, pH 7.4 at 4C for 48hr then rinsed 3 times with 0.1M cacodlylate buffer, pH 7.4 and shipped immersed in the buffer. The maxillae, with teeth, were fixed in phosphate buffered formalin, pH 7.0, stored and shipped at 4C. The calvaria were processed as requested by Dr. Partridge. One foot was skinned and then frozen and shipped frozen. All samples arrived at this laboratory in excellent condition. The proximal tibia and part of the shaft were shipped at 4C by overnight mail to Dr. Doty for processing. Dr. Roberts' samples were similarly shipped by overnight mail. calvaria were shipped by overnight mail to Dr. Partridge for analysis. All tissue arrived at their destination without incident.

The tibial shafts were dehydrated in ethyl ether and embedded undecalcified in polyester casting resin (Chemco, San Leandro, CA). The portion of the tibial shaft immediately proximal to the tibiofibular junction was sawed into 50 micron-thick cross sections with a Gillings-Hamco thin sectioning machine. Sections were mounted on slides and exposed to incident and polarized light.

Techniques used by Dr. Doty in processing his tissues and the data are discussed in detail in Supplemental Report 1: Cosmos 2044: Morphological Studies of Bone and Tendon. The techniques and data from Dr. Roberts and his colleagues will be discussed in detail in Supplemental Report 2: Nuclear volume analysis of osteoblast histogenesis in periodontal ligament cells of Cosmos-1887 rats.

Techniques and data from Dr. Partridge will be discussed in detail in Supplemental Report 3: Collagenase Activity in Rat Calvaria. Techniques and data from Dr. Whelan will be discussed in detail in Supplemental Report 4: Biomechanical Properties of Rat Tarsus Bones.

## **RESULTS**

The body weight of the basal group on Cosmos 2044 was 320±10g (±S.D.) in comparison to the 316±10g of the same group in Cosmos 1187 (Table 1). The flight group from Cosmos 2044 weighed 35g more and were 18d older than the Cosmos 1887 flight rats. The Cosmos 2044 vivarium group weighed 21g more and were 21d older than the Cosmos 1887 vivarium rats. The Cosmos 2044 synchronous group weighed 6g less and were 16d older than the Cosmos 1887 synchronous group.

Visual observations of tibial cross sections under brightfield or polarized light did not show any obvious differences. Area and periosteal perimeter measurements (Table 1) showed no significant changes between any of the flight period experimental groups. The only differences noted were between the basal group and other groups. The basal group had a significantly larger marrow area than the tail-suspended group, a larger periosteal perimeter than the flight group, and a significantly larger marrow perimeter than all other groups.

### DISCUSSION

Data from Cosmos 2044 are difficult to compare with Cosmos 1887 due to the age difference. In spite of the age difference, the basal rats on Cosmos 2044 only weighed 4g more than Cosmos 1887 basals, Cosmos 2044 flight rats gained 16 gms during the flight period vs 9 gained by the Cosmos 1887 rats, the synchronous group on both missions gained essentially the same weight, and the vivarium group on Cosmos 2044 gained 65g vs 25g gained by this group during Cosmos 1887.

The larger bones in the basal group of Cosmos 2044 are an unexplained finding and have not been seen before on any Cosmos mission (Table 1). On Cosmos 1887, area and perimeter measurements

at the tibiofibular junction only showed differences between the synchronous and basal groups with the synchronous bones being larger (Table 1). With the exception of the marrow perimenter in the synchronous and vivarium group, the area and perimenter measurements in the Cosmos 2044 rats were larger than those found in Cosmos 1887. The larger bones on Cosmos 2044 agree with the older age of the rats and the lack of any increase in bone mass during the flight period indicates that the animals were adults and that bone mass was not accumulating rapidly in any group during the 14d flight period, unlike some of the animals in Cosmos 1887. Larger, adult rats may require a longer flight period to demonstrate bone changes particularly in cortical bone since the skeleton is turning over more slowly.

## **ACKNOWLEDGEMENTS**

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TABLE 1

# **COSMOS 2044 PARAMETERS**

GROUP	BASAL	FLIGHT	SYNCHRONOUS	VIVARIUM	TAIL-SUSPENDED
MEASUREMENT					
Body mass, g loading final D	320±10	321±5.2 338±4.6 16±4.3	307±6.8 343±15.7 36±5.8	303±21 363±4.5 65±1.6	303±21 339±21 37±4.6
Bone area, mm <sup>2</sup>	4.79±0.38	4.33±0.19	4.59±0.34	4.61±0.30	4.75±0.51
Marrow area, mm <sup>2</sup>	0.99±0.14	0.87±0.07	0.91±0.06	0.89±0.12	0.82±0.10*
Periosteal perimeter, mm	8.93±0.26	8.36±0.25*	8.60±0.28	8.60±0.21	8.73±0.55
Marrow perimeter, mm	3.76±0.28 n	3.45±0.17*	3.46±0.09*	3.30±0.21*	3.43±0.25*

Data are expressed as mean ± S.D.

\* = significantly different from basal (p<0.05)